Neuraminidase

Source: Cloned from Clostridium perfringens (1) and overexpressed in E. coli at NEB (2).

Supplied in: 50 mM NaCl, 20 mM Tris-HCl (pH 7.5 @ 25°C) and 5 mM Na₂EDTA.

Reagents Supplied with Enzyme:
10X G1 Reaction Buffer

Reaction Conditions:
1X G1 Reaction Buffer:
50 mM Sodium Citrate (pH 6.0 @ 25°C). Incubate at 37°C.

Optimal incubation times and enzyme concentrations must be determined empirically for a particular substrate.

Unit Definition: One unit is defined as the amount of enzyme required to cleave > 95% of the terminal α-Neu5Ac from 1 nmol Neu5Acα2-3Galβ1-3GlcNAcβ1-3Galβ1-4Glc-7-amino-4-methyl-coumarin (AMC), in 5 minutes at 37°C in a total reaction volume of 10 µl.

Unit Definition Assay: Two fold dilutions of Neuraminidase are incubated with 1 nmol AMC-labeled substrate and 1X G1 Reaction Buffer in a 10 µl reaction. The reaction mix is incubated at 37°C for 5 minutes. Separation of reaction products are visualized via thin layer chromatography (3).

Specific Activity: ~200,000 units/mg.

Molecular Weight: 43,000 daltons.

Quality Assurance: No contaminating exoglycosidase or proteolytic activity could be detected.

Quality Controls
Glycosidase Assays: 500 units of Neuraminidase were incubated with 0.1 mM of fluorescently-labeled oligosaccharides and glycopeptides, in a 10 µl reaction for 20 hours at 37°C. The reaction products were analyzed by TLC for digestion of substrate.

Physical Purity: Purified to > 95% homogeneity as determined by SDS-PAGE analysis using Coomassie Blue detection.

No other glycosidase activities were detected (ND) with the following substrates:

β-N-Acetyl-glucosaminidase:
GlcNAcβ1-4GlcNAcβ1-4GlcNAc-AMC ND

α-Fucosidase:
Fucα1-2Galβ1-4Glc-AMC Galβ1-4
(Fucα1-3)GlcNAcβ1-3Galβ1-4Glc-AMC ND

β-Galactosidase:
Galβ1-3GlcNAcβ1-4Galβ1-4Glc-AMC ND

α-Galactosidase:
Galα1-3Galβ1-4Galβ1-3Gal-AMC ND

α-Mannosidase:
Manα1-3Manβ1-4GlcNAc-AMC
Manα1-3Manα1-4Manβ1-4 (Manα1-3)Man-AMC ND

β-Glucosidase:
Glcβ1-4Glcβ1-4Glc-AMC ND

β-Xylosidase:
Xylβ1-4Xylβ1-4Xylβ1-4Xyl-AMC ND

(see other side)
**β-Mannosidase:**
Manβ1-4Manβ1-4Man-AMC  ND

**Endo F₁, F₂, H:**
Dansylated invertase high mannose.  ND

**Endo F₁, F₂:**
Dansylated fibrinogen biantennary.  ND

**PNGase F:**
Fluoresceinated fetuin triantennary.  ND

**Protease Assay:** After incubation of 500 units of Neuraminidase with 0.2 nmol of a standard mixture of proteins in a 20 µl reaction, for 20 hours at 37°C, no proteolytic activity could be detected by SDS-PAGE.

**Note:** This enzyme shows a preference for α2,3 and α2,6 linkages over α2,8 linkages (4).

**References:**